BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

B_1 receptors as a new inflammatory target. Could this B the 1?

Amrita Ahluwalia and Mauro Perretti

The kinins, particularly bradykinin (BK), are important mediators involved in both the initiation and progression of an inflammatory response. The pro-inflammatory effects of kinins are mediated by at least two receptors: the B_2 subtype is expressed constitutively and the B_1 receptor is induced following tissue inflammation and damage. The endogenous ligand for the B_1 receptor is des-arg^{9}BK, a cleavage product of the activity of carboxypeptidases on BK. Activation of B_1 receptors produces a range of pro-inflammatory effects including oedema, pain and promotion of blood-borne leukocyte trafficking. In this article **Amrita Ahluwalia and Mauro Perretti** briefly describe the biology of BK and its receptors, and discuss the possible development of B_1 receptor antagonists as novel anti-inflammatory agents.

The inflammatory response involves a highly complex interplay between multiple factors at humoral, cellular and neuronal levels. One particularly interesting family of inflammatory substances, which plays integral roles at all of these individual levels, is the kinins. The name bradykinin (BK) was coined by Rocha e Silva in 1949 (Ref. 1) and attributed to the substance produced from the activity of trypsin on plasma globulins which caused decreases in blood pressure in dogs and contraction of guinea-pig ileum. In the 1960s, pure BK became available and studies soon followed demonstrating the inflammatory properties of BK. Consequently, its implication as an important mediator in inflammatory processes ensued: the peptide causes vasodilatation and oedema formation, cellular accumulation and pain[2]. These inflammatory effects are mainly mediated by two endogenous kinins, BK and Lys-BK, which are formed by proteolytic cleavage from high- and low-molecular-weight kininogens (hmwKG and lmwKG) by plasma or tissue kallikrein (PKK or TKK), respectively. BK and Lys-BK are then metabolized by kininase I or kininase II (also known as angiotensin converting enzyme). Of particular relevance to the present review is the activity of kininase I to produce des-Arg^{9}BK (DABK) and des-Arg^{10}-Lys-BK (Lys-DABK) from BK and Lys-BK, respectively: it is now clear that, although under normal conditions these metabolites are largely inactive, in certain situations they exhibit biological activity. How can this be explained? Of the two currently known kinin receptors, termed B_1 and B_2, the B_2 receptor is constitutively expressed and mediates many of the actions of BK, whereas the B_1 receptor is normally absent but can be induced under conditions of inflammation. This differential expression of the B_1 receptor explains the difference in activity of the metabolites (described above), but also makes it an exciting potential therapeutic target.

In the acute inflammatory response, plasma protein exudation provides substrate and kallikrein (TKK from infiltrating cells) for the local generation of BK and Lys-BK (Ref. 3). This assures the early availability of these mediators at an inflammatory site and highlights their potential involvement in the manifestation of the complex process of inflammation (see Fig. 1). The role of DABK, or indeed Lys-DABK, in the development and progression of inflammation is only just beginning to be appreciated with the renaissance of interest in the B_1 receptor.

Induction of B_1 receptors: how, where and when

The biological effects of BK are brought about by its interaction with specific G protein-coupled receptors[4,5]. At present, there are two clearly defined and cloned kinin receptors: B_1 and B_2. B_2 receptors are constitutively present on many cell types involved with the inflammatory response: endothelial cells, mast cells[3] and sensory neurones[6]. The natural endogenous ligand for this receptor is BK, with Lys-BK in most cases possessing a lower afffinity for the receptor. In contrast, B_1 receptors are not normally expressed in basal conditions but are induced in situations of stress, such as shock and inflammation. The 'classical' natural ligand for this receptor is DABK, although more recently it has become clear that, in certain human cellular binding assays, Lys-DABK is up to 1000 times more potent than DABK (Ref. 4). This suggests, in humans at least, that Lys-DABK might be the natural ligand at B_1 receptors. Although the endogenous B_1 receptor ligands have no effect at B_2 receptors, BK and Lys-BK bind to human B_1 receptors. For example, BK displays a relatively low affinity (pIC_{50} = 5.7–5.1) for the B_1 receptor in human transfected cell lines[7]. However, Lys-BK has a higher affinity for B_1 than DABK itself. These results indicate that in some situations Lys-BK could mediate its actions through activation of B_1 and not B_2 receptors. As indicated earlier, DABK and Lys-DABK are metabolites of BK and Lys-BK, produced by cleavage of the carboxy-terminal arginine by kininase I. Kininase I identifies a family of two distinct enzymes termed carboxypeptidase N (KI-CPN) and carboxypeptidase M (KI-CPM). KI-CPN is a 280–320 kDa tetrameric complex found in plasma, whereas KI-CPM (a 62 kDa metallopeptidase) is a cell membrane enzyme found in various cells including endothelial cells and fibroblasts[3] (and therefore available locally at the site of inflammation).

The B_1 receptor was first isolated and cloned from a human lung fibroblast cDNA library[4], followed rapidly by the sequences for rabbit[8], mouse[9] and, more recently, the rat (GenBank accession no. U66107). With the cloning

A. Ahluwalia, Lecturer (BHF Intermediate Fellow), Centre for Clinical Pharmacology, UCL, The Rayne Institute, 5 University Street, London, UK WC1E 6JJ, and **M. Perretti,** Senior Lecturer (ARC Fellow), Department of Biochemical Pharmacology, The William Harvey Research Institute, Charterhouse Square, London, UK EC1M 6BQ.

0165-6147/99/$ – see front matter PII: S0165-6147(99)01321-8

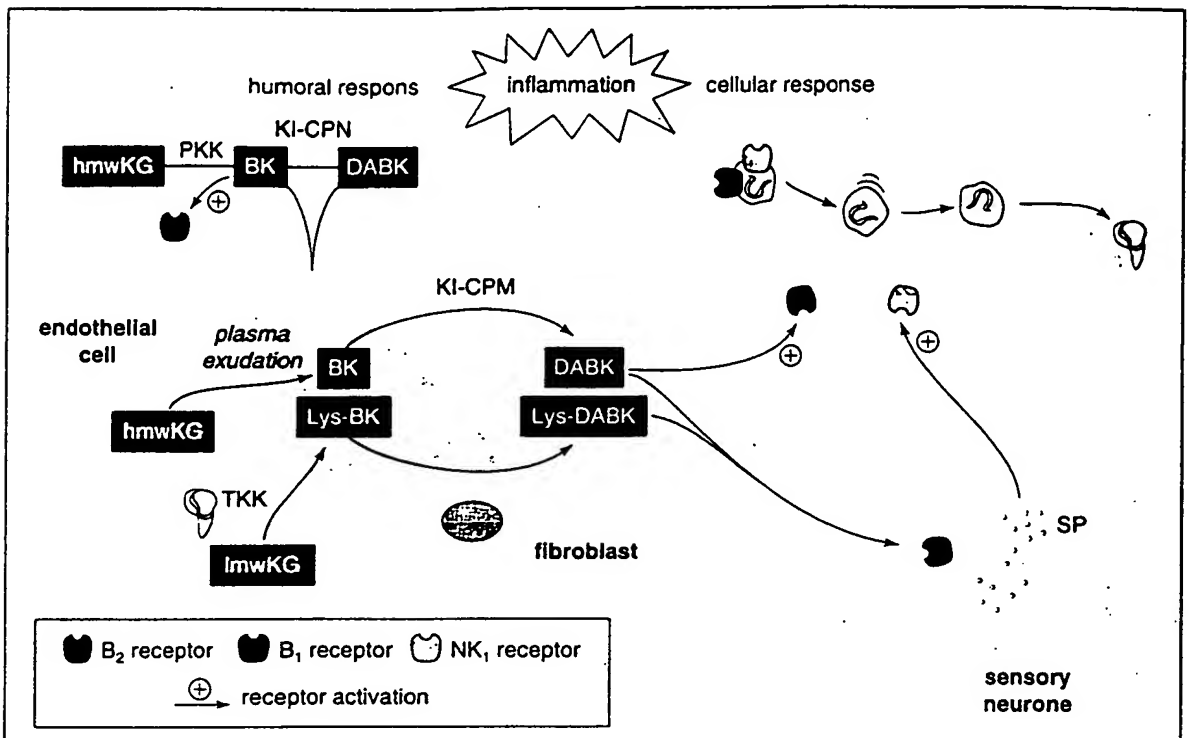

Fig. 1. Schematic diagram of the synthesis and possible effector sites of des-Arg[9]bradykinin (DABK) in inflammation. The intravascular formation of bradykinin (BK) occurs by the action of plasma kallikrein (PKK) on high-molecular-weight kininogen (hmwKG). Kininase I-carboxypeptidase N (KI-CPN) produces DABK from BK. During the plasma exudation phase of the inflammatory response, BK synthesized in plasma might act on endothelial B_2 receptors to potentiate oedema formation directly. During the humoral response, plasma-borne kinins as well as their precursor can reach the site of inflammation. Locally, kinins can be synthesized by the action of tissue kallikrein (TKK), provided by infiltrated leukocytes, on (1) low-molecular-weight kininogen (lmwKG) leading to Lys-BK formation, and/or (2) extravasated hmwKG leading to further synthesis of BK. Endothelial cell (ec) and fibroblast (fb) kininase I-carboxypeptidase M (KI-CPM) converts BK to DABK as well as Lys-BK to Lys-DABK (Des-Arg[10]-Lys-bradykinin). DABK and Lys-DABK act on B_1 receptors expressed on endothelial cells and possibly also on sensory neurones to activate the process of leukocyte trans-endothelial migration. Kinin-mediated leukocyte migration might involve, as an intermediate, release of substance P (SP) for sensory neurones, which will activate endothelial NK_1 receptors.

of the receptor, several groups have begun to determine the signal transduction mechanisms as well as to dissect the molecular mechanisms involved in induction of the receptor. Interestingly, it seems that the inflammatory transcription factor, nuclear factor κB (NF-κB), has a role not only as a mediator of the inflammatory effects of B_1 receptor activation but is also involved in its induction[10,11]. The identification of NF-κB binding domains[11] in the promoter region of the gene implies that any of the multitude of inflammatory mediators shown to possess the capacity to stimulate increases in NF-κB expression could, in turn, stimulate expression of the B_1 receptor. One such mediator is BK itself[12]; indeed, there is indirect evidence that activation of B_2 receptors might induce B_1 receptors. Single intradermal application of BK, in rats *in vivo*, caused oedema formation whereas DABK did not. Repeated administration of BK over 24 h resulted in an apparent tachyphylaxis of the response associated with a presentation of an oedema response to DABK when injected into the same site[13]. However, in these studies it is unclear whether it is the inflammatory response stimulated by BK application, rather than a specific effect of B_2 receptor activation or desensitization that is the stimulus for the apparent induction of the B_1 receptor. The availability of cellular expression systems for kinin receptors has provided valuable information about the nature and indeed differences between the two types of receptor. Particularly interesting is the finding that, whereas the B_2 receptor suffers rapid internalization and hence apparent desensitization to BK (i.e. loss of sensitivity to BK) the B_1 receptor, once induced, does not[14]. Whether the downregulation of B_2 receptors is a stimulus for B_1 receptor induction is unclear. Of course, the next development that would help to probe these relationships further is the development of B_1 receptor knockouts. B_2 receptor knockout mice have already been produced and studies have indicated the importance of this receptor both in maintenance of normal blood pressure and development of nociception in a model of inflammatory hyperalgesia[15,16].

The first demonstration of a B_1 receptor-mediated response was provided in 1978 by Regoli and co-workers, who showed contraction of rabbit aortic rings to DABK only following long term *in vitro* incubation[17] or pretreatment of animals with lipopolysaccharide or cytokines[18]. This assay system was then used as the basis for the pharmacological characterization of the kinin receptors, also made possible by the development of specific antagonists for both B_1 and B_2 receptors. It was in the late

1970s that the first selective kinin receptor antagonist des-Arg^9[Leu]^{8}BK (DALBK)[19] was described and used to identify the B_1 receptor. This drug antagonized the actions of DABK on the rabbit aorta without affecting many of the biological effects of BK (Ref. 19). It was suggested that those effects insensitive to the antagonist were due to activation of a separate kinin receptor subtype, which was termed B_2. Interest in the B_1 receptor waned when several studies followed showing the partial agonist behaviour of DALBK and it has only been as a result of the renewed interest in B_1 receptors that attempts to produce more selective compounds have been pursued. Among the best of the peptidic antagonists is B9958, a compound highly selective for the human B_1 receptor (see Ref. 7 for an extensive review of the currently available B_1 receptor blockers). To date there are no nonpeptidic B_1 receptor antagonists. Unlike the situation with the B_1 antagonists, it was not long after the initial description of DALBK before selective B_2 antagonists were developed. The first antagonists produced were peptidic in nature and prone to enzyme attack and therefore had short half-lives[20]. A significant advance came with the generation of the stable peptidic B_2 receptor-selective antagonist, Hoe140 (icatibant[21]) and more recently the development of nonpeptidic blockers. The first nonpeptidic antagonist to be described was WIN64338, shown to be an effective antagonist in guinea-pig tissues but with little activity in human tissues[7]. More recently, Fujisawa has developed a compound (FR173657) that displays potent activity across the species and is orally active[22]. Importantly, these antagonists also provide an invaluable tool when attempting to discern the role of kinins and their respective receptor subtypes in both physiological and pathological processes. Indeed, using these antagonists, it is now clear that B_2 receptors are involved in mounting an inflammatory response. The first demonstration of such an effect came in 1991, where it was shown that Hoe140 dose-dependently inhibited the oedema response to carrageenin injected into the rat paw[23].

Relevance of B_1 receptors to inflammation

It is now clear that responses to DABK are, in the large part, observed following a pathological insult, and experimentally this has been very well demonstrated using models of sepsis and nociceptive inflammation[24]. In both cases, a rise in local or circulating cytokine levels, particularly interleukin 1 (IL-1), has been implicated in the process of induction. There is support for the concept that not only are B_1 receptors upregulated by specific inflammatory stimuli, but that there is also an increase in the levels of DABK produced[25]. This elevation could be due to an increase in the activity of CPM, an enzyme involved in the synthesis of DABK, as endotoxin infusion in pigs is associated with an increase in the expression of this enzyme[26]. Thus, during inflammation it is apparent that both agonist and receptor are upregulated. These lines of evidence suggest that B_1 receptor activation might be functionally involved in mediating the cardinal signs of inflammation. Indeed, there are several lines of evidence that support such a hypothesis. Below we discuss the possible role of B_1 receptor activation in certain aspects of inflammation with particular emphasis on a novel site of action: the cellular component of the inflammatory responses.

Humoral: oedema

Plasma-protein extravasation is a prominent feature of the acute inflammatory response and can be induced by a wide range of mediators acting on endothelial cells in post-capillary venules. This process is thought to provide both preformed kinin proteins, synthesized due to activation of the PKK system, and TKK (the source of this enzyme being infiltrating leukocytes for instance)[27] for the local synthesis of kinins. TKK acts on lmwKG found in the extravascular space, for example on fibroblasts. In these ways, the local kinin concentration is elevated at the site of inflammation. An interesting postulate is that during an acute inflammation, plasma kinin levels rise resulting in activation of B_2 receptors situated on endothelial cells in the vasculature. This in turn would stimulate an increase in venular permeability providing all of the necessary components for elevated kinin synthesis, including the endogenous B_1 agonists, at the inflammatory site. Indeed, there is evidence to support the concept that DABK levels are elevated in inflammation[28]. This enhanced, local kinin synthesis along with induced expression of B_1 receptors could then contribute to exacerbation of the inflammation, including increased plasma protein extravasation. Intradermal or intraplantar administration of DABK, in normal rats, has been shown to have little or no oedema-inducing activity[13], whereas in other studies, appreciable effects following intra-articular injection in the rat[29] or intrathoracic and intraplantar injection in mice have been described[30]. In all of the above systems, the oedema responses to DABK were significantly enhanced following inflammatory stimulus, and as mentioned earlier in some cases with respect to the induction of oedema, desensitization to BK is associated with a compensatory induction of oedema responses to previously inactive DABK (Ref. 13). Irrespective of the mechanism of induction, these results suggest that the kinin–kallikrein system can operate both at the acute and chronic stages of inflammation, B_2 receptors mediating the former and B_1 the latter. Indeed, in the main, B_1 receptors seem to be upregulated only during the chronic stages of inflammation, and this is possibly the point at which B_2 receptors have become desensitized. Such a hypothesis is supported by the finding that treatment of animals with a mixed B_1/B_2 receptor antagonist improves mortality in a model of sepsis[31], whereas there is some controversy over the efficacy of selective receptor antagonists.

Neuronal: inflammatory pain

The first observations demonstrating a link between B_1 receptor activation and nociceptive inflammation were

described by Perkins and his co-workers, who showed that pain associated with chronic inflammation is blocked by DALBK (Ref. 32). Although these studies clearly demonstrate the involvement of B_1 receptors in integration of the nociceptive signal associated with inflammatory pain, the exact cellular localization of the receptor remained unclear to this group. They did, however, tentatively suggest that the sensory neurone might be the site of expression, because hyperalgesia is essentially neurogenic in nature and sensitive to agents that modify sensory C-fibre activity (e.g. capsaicin). These fibres contain the pro-inflammatory peptides substance P (SP) and calcitonin-gene related peptide (CGRP)[33]. There is evidence however, that B_1 receptor activation by DABK results in the release of hyperalgesic prostanoids[32].

Cellular response

In 1996 we reported the clear involvement of induced B_1 receptors and sensory neurones in IL-1β-induced cellular migration and accumulation[34]. The intense neutrophil migration in response to cytokine application (maximal at 4 h) was reduced by co-injection of DALBK, whereas Hoe140 had no effect. Moreover, following IL-1β administration, previously inactive DABK then acquired the capacity to induce neutrophil accumulation itself. In view of the pivotal role played by this cytokine in the development of the host inflammatory response, we proposed that induction and activation of B_1 receptors could be of considerable importance. The next obvious question was 'where are B_1 receptors located and how did this relate to attraction of neutrophils during the host response?' We demonstrated that neutrophils accumulated at the site of IL-1β injection did not respond to DABK *ex vivo*. (Indeed, it now appears that if a kinin receptor is expressed on neutrophils that it is of the B_2 receptor subtype[35].) We postulated, therefore, that the receptors may be expressed on sensory C-fibres because IL-1β-induced neutrophil accumulation into the murine air pouch is attenuated by: (1) chronic capsaicin treatment; (2) substance P tachykinin NK_1 receptor antagonists; and (3) CGRP receptor antagonism. Importantly, block of both receptors almost abolished (≥80% reduction) DABK-induced cell accumulation in cytokine-pretreated animals. This link between B_1 receptors and sensory C-fibres was substantiated by Vianna and Calixto who showed that intrapleural injection of DABK resulted in an activation of the host acute inflammatory response[30]. This response consisted not only of a rapid increase in vascular permeability, and consequent oedema formation, but also of a delayed (maximal at 4 h) accumulation of neutrophils (cellular response). These authors also confirmed the involvement of sensory neuropeptides as NK_1, NK_2 and NK_3 receptor antagonists attenuated the DABK-induced neutrophil and monocyte accumulation into the murine pleural cavity. Furthermore, this group showed an instrumental role for endogenous NO in that DABK-induced cell migration was attenuated by NO synthase inhibitors. It is clear from both our study[34] and that of Vianna and Calixto[30] that B_1 receptors are present either on the sensory nerve or on an accessory cell which in turn activates the sensory nerve. The end-point in either case is the release of neuropeptides in the inflammatory microenvironment with potentiation of the cellular response.

Concluding remarks

The picture emerging from several recent studies is that the physiological tonic actions of kinins involve activation of B_2 receptors. However, in pathological conditions, it is not only B_2 receptors that mediate the inflammatory actions of kinins: B_1 receptors are induced *de novo* and might be activated by the specific endogenous agonist DABK, which is elevated in inflammatory exudates. In the inflammatory scenario, the effects of B_1 receptor activation are often qualitatively similar to those of BK and B_2 receptor activation. Interestingly, presentation of responses to endogenous B_1 agonists is often associated with loss of sensitivity to BK. This could represent a mechanism whereby the kinin–kallikrein system contributes to the change of an acute inflammatory situation to the chronic arena characterized by an inflammatory cellular infiltrate.

The host inflammatory response is the result of a complex series of integrated phenomena: arteriolar dilatation, increased vascular permeability, leukocyte interaction with the endothelium followed by diapedesis and migration to the site of insult. It is clear, as stated by Gallin *et al.*[36], that 'information concerning the mechanisms whereby inflammatory cells accumulate in tissues ... should provide clues for developing more rational forms of therapy'. The studies described here strongly propose B_1 receptors as a novel target for development of anti-inflammatory drugs with a potent action on leukocyte recruitment.

References
1 Rocha e Silva, M., Beraldo, W. T. and Rosenfeld, G. (1949) *Am. J. Physiol.* 156, 261–273
2 Lewis, G. P. (1963) *Ann. New York Acad. Sci.* 104, 236–249
3 Bhoola, K. D., Figueroa, C. D. and Worthy, K. (1992) *Pharmacol. Rev.* 44, 1–79
4 Menke, J. G. *et al.* (1994) *J. Biol. Chem.* 269, 21583–21586
5 Hess, J. F., Borkowski, J. A., Young, G. S., Strader, C. D. and Ransom, R. W. (1994) *Mol. Pharmacol.* 45, 1–8
6 Steranka, L. R. *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 3245–3249
7 Regoli, D., Allogha, S. N., Rizzi, A. and Gobeil, F., Jr (1998) *Eur. J. Pharmacol.* 348, 1–10
8 MacNeil, T. *et al.* (1995) *Biochim. Biophys. Acta* 1264, 223–228
9 Pesquero, J. B. *et al.* (1996) *Biochem. Biophys. Res. Commun.* 220, 219–225
10 Ni, A., Chao, L. and Chao, J. (1998) *J. Biol. Chem.* 273, 2784–2791
11 Schanstra, J. P. (1998) *J. Clin. Invest.* 101, 2080–2091
12 Pan, Z. K. (1996) *J. Clin. Invest.* 98, 2042–2049
13 Campos, M. M. and Calixto, J. B. (1995) *Br. J. Pharmacol.* 114, 1005–1013
14 Austin, C. E. *et al.* (1997) *J. Biol. Chem.* 272, 11420–11425
15 Madeddu, P. *et al.* (1997) *Circulation* 96, 3570–3578
16 Boyce, S. *et al.* (1996) *Immunopharmacology* 33, 333–335
17 Regoli, D., Marceau, F. and Barabe, J. (1978) *Can. J. Physiol. Pharmacol.* 56, 674–677
18 Regoli, D., Marceau, F. and Lavigne, J. (1981) *Can. J. Physiol. Pharmacol.* 71, 105–115
19 Regoli, D. and Barabe, J. (1980) *Pharmacol. Rev.* 32, 1–46
20 Vavrek, R. and Stewart, J. M. (1985) *Peptides* 6, 161–164
21 Hock, F. J. *et al.* (1991) *Br. J. Pharmacol.* 769, 769–773
22 Inamura, N. *et al.* (1997) *Can. J. Physiol. Pharmacol.* 75, 622–628

23 Wirth, K. *et al.* (1991) *Br. J. Pharmacol.* 102, 774–777
24 Marceau, F. (1997) in *The Kinin System* (Farmer, S. G., ed.), pp. 143–156, Academic Press
25 Raymond, P. *et al.* (1995) *J. Immunol. Methods* 180, 247–257
26 Schremmer-Daninger, E., Offner, A., Siebeck, M. and Roscher, A. A. (1998) *Biochem. Biophys. Res. Commun.* 243, 246–252
27 Figueroa, C. D., Maciver, A. G. and Bhoola, K. D. (1989) *Br. J. Haematol.* 72, 321–328
28 Burch, R. M. and DeHaas, C. (1998) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 342, 189–193
29 Cruwys, S. C. *et al.* (1994) *Br. J. Pharmacol.* 113, 940–944
30 Vianna, R. M. J. and Calixto, J. B. (1998) *Br. J. Pharmacol.* 123, 281–291
31 Whalley, E. T. *et al.* (1992) *Agents Actions* 38, 482–487
32 Dray, A. and Perkins, M. (1993) *Trends Neurosci.* 16, 99–104
33 Davis, C. L. *et al.* (1996) *Br. J. Pharmacol.* 118, 1469–1476
34 Ahluwalia, A. and Perretti, M. (1996) *J. Immunol.* 156, 269–274
35 Rajasekariah, P., Warlow, R. S. and Walls, R. S. (1997) *Biochem. Mol. Biol. Int.* 43, 279–290
36 Gallin, J. I., Goldstein, I. M. and Snyderman, R. (1992) in *Inflammation: Basic Principles and Clinical Correlates* (Gallin, J. I., Goldstein, I. M. and Snyderman, R., eds), pp. 1–4, Raven Press

Acknowledgements
AA is supported by the British Heart foundation and MP by the Arthritis Rheumatism Council.

Chemical names

BN9958: Lys-Lys-[Hyp^3,Cpg^5,D-Tic^7,Cpg^8]desArg9BK

FR173657: (*E*)-3-(6-acetamido-3-pyridyl)-*N*-(*N*-[2,4-dichloro-3[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-*N*-methylaminocarbonyl-methyl)acrylamide

Hoe140: [D-Arg-Arg-Pro-Hyp-Gly-(β-2-thienyl)-Ala-Ser-DTic-Oic-Arg

WIN64338: [[4-[[2-[[bis(cyclohexylamino)methylene]-amino]-3-(2-naphthyl)-1-oxopropyl]amino]phenyl]-methyl]tributyl phosphonium chloride monohydrochloride

Emerging functions for neuropeptide Y_5 receptors

Angela Bischoff and Martin C. Michel

The Y_5 subtype of neuropeptide Y (NPY) receptors has raised considerable interest as a mediator of NPY-stimulated food intake, but with the advent of recent data, this hypothesis has come into question. Moreover, Y_5 receptor-selective drugs might not be specific for food intake because additional functions in the central and peripheral nervous systems, including endogenous anti-epileptic activity, attenuation of morphine withdrawal symptoms, enhancement of diuresis and natriuresis, lowering of blood glucose and reduction of acetylcholine release in the ileum, have recently been reported to occur via Y_5-like receptors. Given that mRNA for the cloned Y_5 receptor is apparently restricted to the CNS, **Angela Bischoff and Martin Michel** discuss the possible existence of additional NPY receptor subtypes with Y_5-like recognition features and their presence in peripheral tissues.

A. Bischoff, Postdoctoral researcher, and **M. C. Michel,** Professor of Pharmacology, Department of Medicine, Nephrology Lab. IG 1, Klinikum Essen, Hufelandstrasse 55, 45122 Essen, Germany.

The putative neurotransmitter neuropeptide Y (NPY) and the hormones peptide YY (PYY) and pancreatic polypeptide (PP) are members of a family of peptides[1] that act on a common set of receptors designated NPY receptor (Ref. 2). Although initial functional studies with agonists indicated the presence of two NPY receptor subtypes, Y_1 and Y_2 (Ref. 3), molecular cloning has identified three additional subtypes, namely Y_4, Y_5 and y_6 (Ref. 2). Although the Y_5 receptor was identified primarily using molecular cloning, a number of Y_5 receptor-mediated functions have been revealed; these will be the subject of this review.

Y_5 receptor identification

The cDNAs for the Y_5 receptor have been cloned from rats, mice and humans[4–6]; their corresponding GenBank accession numbers are U65078, AF022948 and U56079, respectively. The genes encoding the Y_5 receptor reside on human chromosome 4q31–q32 (Refs 4, 5, 7) and mouse chromosome 8 (Ref. 6), whereas the Y_1 receptor genes are found in the same locations but in opposite orientation[4–7]. Northern blotting has detected Y_5 receptor mRNA in rat and murine brain, but not in several peripheral tissues (heart, spleen, lung, liver, skeletal muscle and kidney) except for a weak signal in the rat testis[4–6]. Within the rat brain, *in situ* hybridization has detected Y_5 receptor mRNA in the paraventricular hypothalamic nucleus, the lateral hypothalamus and the arcuate nucleus[4], which is consistent with a role for Y_5 receptors in the regulation of food intake (see below).

In keeping with other NPY receptor subtypes, the cloned rat Y_5 receptor couples to inhibition of cAMP accumulation when expressed in HEK293 cells[4]. In radioligand binding and cAMP accumulation studies, NPY, PYY, [Pro^{34}]-substituted analogues and long C-terminal fragments thereof (e.g. NPY_{2-36}, PYY_{3-36}) have high potency at the Y_5 receptor, while the latter have low potency at the Y_1 receptor. Short C-terminal fragments (e.g. NPY_{13-36}) and the Y_1 receptor-selective antagonist BIBP3226 have a low affinity for the Y_5 receptor[4,5]. Interestingly, rat PP has very low affinity for the rat and human Y_5 receptor, whereas human and bovine PP has affinities similar to those of NPY and PYY (Refs 4, 5). Although the Y_2 receptor has a high affinity for short C-terminal fragments of NPY and PYY, e.g. NPY_{13-36}, the Y_5 receptor has low affinity. Distinguishing the Y_5 receptor from the Y_4 receptor is also possible as the affinity of NPY and PYY is similar or even greater than that of PP at Y_5 receptors but is much lower than that of PP at the Y_4 receptor. Thus, the Y_5 receptor can be discriminated pharmacologically from other NPY receptor subtypes[2].

The identification of Y_5 receptor-mediated function presently relies on three main approaches, each of which